

AMENDMENTS TO THE CLAIMS:

Claims 1-80, 82, 84-86 and 93-99- are canceled without prejudice or disclaimer. The following is the status of the claims of the above-captioned application, as amended.

Claim 1-80. (Previously cancelled.)

Claim 81. (Previously presented.) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca²⁺ -dependency and specific activity, wherein said parent alpha-amylase has a sequence of at least 70% homology to the sequence of SEQ ID No: 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.3) using default values for GAP penalties, said method comprising

(a) generating a three-dimensional model of a parent alpha-amylase structure, utilizing data from Appendix 1 and a computer programmed for generating said model from said data;

(b) identifying in said three-dimensional parent alpha-amylase structure generated in step (a) at least one amino acid residue or at least one structural part; wherein an alteration in said at least one amino acid residue or said at least one structural part is predicted to result in said altered property;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue or at least one structural part identified instep (b); and

(d) expressing the modified nucleic acid of step (c) in a host cell to produce said variant alpha amylase.

Claim 82. (Cancelled.)

Claim 83. (Previously presented.) The method according to claim 81, wherein said three-dimensional alpha amylase structure has an A domain, a B domain and a C domain, wherein said A domain has an amino acid sequence corresponding to residues 1-103 and 206-395 of SEQ ID NO: 2; said B domain has an amino acid sequence corresponding to residues 104-205 of SEQ ID

NO:2 and said C domain has an amino acid sequence corresponding to residues 396-483 of SEQ ID NO:2.

Claims 84-86. (Cancelled.)

Claim 87. (Previously presented.) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase wherein said altered property is increased calcium binding affinity, said method comprising

(a) generating a model of a three-dimensional structure of a parent alpha-amylase, using the coordinates of the three-dimensional structure of SEQ ID NO:13 depicted in Appendix 1 and a computer programmed for generating a model structure, said parent alpha-amylase having at least 70% homology to SEQ ID NO:13.

(b) utilizing said three-dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure at least one amino acid residue or structural part within 10Å from a calcium binding site;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue identified in step (b); and

(d) expressing the modified nucleic acid in a host cell to produce said variant alpha-amylase.

Claim 88. (Previously presented.) A method according to claim 87, wherein the variant has a decreased calcium ion dependency of enzymatic activity or stability.

Claim 89. (Previously presented.) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase wherein said altered property is selected from the group consisting of pH optimum and the enzymatic activity at a given pH, said method comprising

(a) generating a model of a three-dimensional structure of a parent alpha-amylase, using the coordinates of the three-dimensional structure of SEQ ID NO:13 depicted in Appendix 1 and a computer programmed for generating a model structure, said parent alpha-amylase having at least 70% homology to SEQ ID NO:13;

(b) utilizing said three-dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure at least one amino acid residue or structural part within 15Å from an active site residue;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue identified in step (b); and

(d) expressing the modified nucleic acid in a host cell to produce said variant alpha-amylase.

Claim 90. (Previously presented) The method according to claim 89, wherein the variant has an altered pH optimum relative to the parent.

Claim 91. (Previously presented.) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase wherein said altered property is selected from the group consisting of substrate specificity, substrate binding and substrate cleavage pattern said method comprising

(a) generating a model of a three-dimensional structure of a parent alpha-amylase, using the coordinates of the three-dimensional structure of SEQ ID NO:13 depicted in Appendix 1 and a computer programmed for generating a model structure said parent alpha-amylase having at least 70% homology to SEQ ID NO:13;

(b) utilizing said three-dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure the substrate binding site;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids of the substrate binding site identified in step (b); and
expressing the modified nucleic acid in a host cell to produce said variant alpha amylase.

Claim 92. (Previously presented.) The method according to claim 91, wherein the variant has a reduced ability to cleave a substrate close to a branching point relative to the parent.

Claims 93.-99 (Cancelled.)